

Morphology of Females and Cysts of *Globodera tabacum tabacum*, *G. t. virginiae*, and *G. t. solanacearum* (Nemata: Heteroderinae)¹

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Abstract: Detailed morphological comparisons with light and scanning electron microscopy were made of white females and cysts of several isolates of *Globodera tabacum* ssp. *tabacum* (GTT), *virginiae* (GTV), and *solanacearum* (GTS). Observations focused on body shape, anterior region including head shape, lip pattern, stylet morphology, and the terminal area in females; and body shape and terminal area of cysts. The most useful characters to separate the three subspecies were forms of the female body, cyst, stylet knobs, tail region, perineal tubercles, anal-fenestral ridge patterns, and the distinctiveness of the anus. GTT is characterized by having round females and cysts, sharply back sloped stylet knobs, clumped perineal tubercles in the vulval region, tight parallel ridges in the cyst anal-fenestral region, and a uniformly conoid tail region. GTV is characterized by its ovoid to ellipsoid female and cyst shape, the "Dutch shoe" shape of the dorsal stylet knob, the more dispersed perineal tubercles, a maze-like pattern of ridges in the anal-fenestral region, and an indistinct anus. GTS is characterized by its ovoid to ellipsoid female and cyst shape, moderately backward sloped stylet knobs, more widely separated ridges, a distinct anus, and a usually crescent shaped tail region. Much variability in shape and patterns is visible among all the isolates of the different subspecies. Tubercles in the neck, as well as bullae, are reported, and their taxonomic value is discussed.

Key words: Cyst, *Globodera tabacum tabacum*, *G. t. solanacearum*, *G. t. virginiae*, juvenile, light microscopy, morphology, nematode, scanning electron microscopy, stylet, subspecies, terminal area, variability.

The tobacco cyst nematodes (TCN) are grouped into three sub-species: *Globodera tabacum tabacum* (Lownsbery & Lownsbery, 1954) Behrens, 1975, (5) (GTT), *G. t. virginiae* (Miller & Gray, 1968) Behrens, 1975, (8) (GTV), and *G. t. solanacearum* (Miller & Gray, 1972) Behrens, 1975 (9) (GTS). GTV and GTS primarily occur in Virginia (7), and GTT is limited to Connecticut and Massachusetts (7). Recent observations by light (LM) and scanning electron microscopy (SEM) of second-stage juveniles (J2) and males of the TCN complex revealed no morphological characters that were useful to separate the three subspecies (12). Morphological variability among isolates and subspecies was extremely low for all features examined. Morphometrics of J2 and males were also useless for iden-

tification of subspecies, with the possible exception of male tail length (12).

Traditionally the cyst has been used for identification and taxonomy (1). The terminal area containing the anus and vulva was used to classify *Globodera* species (3,4, 14); however, white females have not been utilized frequently. The only morphological studies of females of the TCN complex, besides the original descriptions, were done by Green (3) and Mulvey (14).

The purpose of this paper is to evaluate the morphological variability of the white females and cysts and to search for new and reliable characters of the three subspecies, GTT, GTV, and GTS. Preliminary observations have shown some variability within each subspecies (11).

MATERIALS AND METHODS

Isolates of TCN used in this study are listed in Table 1. All populations were reared on 'Rutgers' tomato (*Lycopersicon esculentum* L.) in the greenhouse in either 10- or 15-cm clay pots with a 2:1 steam sterilized top soil to sand mix. After 60-70 days, females and cysts were extracted by Cobb's decanting and sieving method. Fe-

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TABLE 1. Isolates of the tobacco cyst nematode complex, *Globodera tabacum* ssp. *tabacum* (GTT), virginiae (GTV), and *solanacearum* (GTS) used in this study.

Isolate	Location	County, state	Origin
GTT-1 (type locality)	Hazardville	Hartford, CT	P. M. Miller
GTT-2	Windsor	Hartford, CT	P. M. Miller
GTT-3	Windsor	Hartford, CT	J. LaMondia
GTT-4	Windsor	Hartford, CT	J. LaMondia
GTT-5	Enfield	Hartford, CT	J. LaMondia
GTV-1	Horton farm	Suffolk, VA	L. I. Miller
GTV-1-X	Horton farm	Suffolk, VA	M. Mota/J. D. Eisenback
GTV-4	93A	Suffolk, VA	L. I. Miller
GTV-6	125A	Suffolk, VA	L. I. Miller
GTV-8	H. N. Williams	Suffolk, VA	L. I. Miller
GTV-11 (type locality)	Standard 24	Suffolk, VA	L. I. Miller
GTS-1	Fisher-Nottoway	Nottoway, VA	L. I. Miller
GTS-3	Irby	Amelia, VA	L. I. Miller
GTS-5	Lynch	Amelia, VA	L. I. Miller
GTS-8	Smith	Amelia, VA	L. I. Miller
GTS-10 (type locality)	Watkins	Amelia, VA	L. I. Miller
GTS-12	D-132	Dinwiddie, VA	Plant Dis. Clinic, Virginia Tech

males were immediately processed for LM and SEM to minimize cement formation around the head. All images were recorded on Polaroid type 55 film.

For LM, females were sequentially fixed in a 1:1 (v:v) mixture of 2% glutaraldehyde and 1% formalin for at least 24 hours. The anterior portion of the body was severed and placed in a drop of water on a thickly ringed slide and covered with a coverslip. Specimens were photographed through a planapochromatic, bright field, compound microscope. The cyst terminal area was cut in 45% lactic acid, transferred to glycerin jelly on a microscope slide, topped with a coverslip, and sealed with nail polish. For SEM, females were placed in a Bureau of Plant Industry (BPI) dish with 10 drops of tap water at 4 C for approximately 15 minutes (2). Two drops of 4% glutaraldehyde was added every 10 minutes. After fixation was completed, specimens were kept in a refrigerator (4–5 C) for 24–48 hours, followed by three rinses with 0.1 M sodium cacodylate buffer (pH 7.2) within a 15-minute period. The specimens were postfixed under a fume hood in 2% osmium tetroxide, kept for 8–48 hours in a refrigerator, and rinsed three times with buffer within a 15-minute

period. Specimens were stored in a desiccator overnight, mounted on SEM stubs with double sticky tape with the head region vertical, sputter-coated with 20 nm of gold–palladium, and observed with a Philips 505 SEM operating at 20 kV with a 20–50 nm spot size. For cysts, the terminal areas were cut in 45% lactic acid and placed directly on a SEM stub with double sided sticky tape, convex side up. To observe the internal morphology of the cyst wall, some specimens were mounted concave side up. Specimens were sputter-coated as described. Stylets were extracted by cutting off the anterior portion of the female body in 0.01–0.05% sodium hypochlorite (10), in which the stylet remained intact while the surrounding tissues dissolved. Some specimens required higher concentrations of bleach. The stylet was cleaned and placed in the central area of the dissecting chamber with a dental root canal file, using a stereoscope at $\times 60$. The stylet was attached and fixed to the cover slip with one drop of 2.5% formalin or tap water every 2 minutes until the sodium hypochlorite was removed. Excess formalin or water was drained from the coverslip with either filter paper or a micropipette, and the stylet was air-dried.

Specimens were stored in a desiccator overnight, mounted on SEM stubs with double sticky tape, sputter-coated, and observed as described.

OBSERVATIONS

Female (Figs. 1–5): Body shape varies from round to ovoid or elliptical, as in cysts. Round females are most frequent among the GTT isolates, whereas those of GTV and GTS are mostly ovoid (Fig. 1A) or ellipsoid. The surface of the female body is covered by cuticular ridges. As in cysts, these ridges are transverse or annulated in the neck and head regions (Figs. 1E,F), zig-zag in the midbody of most specimens, and whorled around the terminal region containing the anus and vulva (Fig. 1D).

The conical neck region is quite variable in size (Figs. 1E,2C). Two or three annules are prominent in the head when viewed laterally with LM. These annules correspond to the oral disk, the fused lips, and perhaps an additional body annule (Fig. 2D). The stomatal opening is centered on the oral disk (Fig. 3C). The oral disk is typically an oblong rectangle in all three subspecies and usually has sharp margins; however, in some specimens the margins are rounded (Fig. 3A). Below the oral disk, the lateral and submedial lips are typically fused. Fusion of the lips can result in the formation of a single hexagonal annule (Fig. 4D). Amphidial openings appear as slits in the lateral lips but frequently are obscured by amphidial exudates (Fig. 3B,D). Lateral and submedial lips can also have irregular margins or cusps (Fig. 4A,C;5A,B), which can be rounded (Fig. 5A,B) or pointed (Fig. 4A,B). Neck annules are continuous (Fig. 5), fragmented, or transformed into tubercles (Fig. 3B,D). Tubercles vary in size but are not present in all specimens. The excretory pore is clearly at the base of the neck (Fig. 1F, arrow). It is contained within a depression in the cuticle.

The stylet is robust with an elastic cone about half the total length, a cylindrical shaft, and three stout basal knobs (Fig.

1K). The dorsal esophageal gland opening (DEGO) is 4–8 μm below the basal knobs (Fig. 1K). The basal knobs in GTT slope sharply posteriorly (Fig. 2B), but in GTV the dorsal knob is typically curved anteriorly like a “Dutch shoe” (Fig. 2F). The basal knobs in GTS are similar to those of GTT but not as sharply sloped posteriorly (Fig. 2J). The shape of the stylet knobs is variable, and some individuals of one subspecies resemble that of another subspecies (Fig. 2C,D,G,H,K,L). Several small vesicles surround the lumen lining between the DEGO (Fig. 1K) and the median bulb in a few specimens of GTT (Fig. 1L). Longitudinal grooves frequently are observed on the stylet shaft of all subspecies in SEM (Fig. 2A,E,I).

The cuticular ridges of the terminal region have a circular whorled pattern (Fig. 1D). The vulval slit and the vulval region are flanked by two vulval crescents made up of perineal tubercles (Fig. 6). These tubercles appear to be more clumped in the GTT isolates (Fig. 6A) and more discrete and dispersed in GTV and GTS (Figs. 6B,D). The vulval region varies in shape but is generally ovoid to elliptical in all three subspecies. It may be more rounded and compressed (Fig. 6A) or more elongated (Fig. 6B). No clear differences are visible in the terminal area of the female that permit differentiation of the three subspecies, except for the more clumped tubercles in GTT.

Cysts (Figs. 7, 8): As in the female, cysts may be rounded in GTT or elliptical to ovoid in GTV and GTS (Fig. 1B–D). The cuticular ridges also display similar patterns, but are more distinct. The neck region has transverse annulations (Fig. 1F), the midbody has zig-zag markings (Fig. 1C), and the terminal area has a whorled pattern (Fig. 1D). At the base of the neck, a transition from zig-zag to the annulated pattern may be visible. Transverse striation at midbody is present in rare instances (Figs. 1D, 3A).

Most observations of the terminal area were made with SEM because of the difficulty of discerning the ridges and grooves

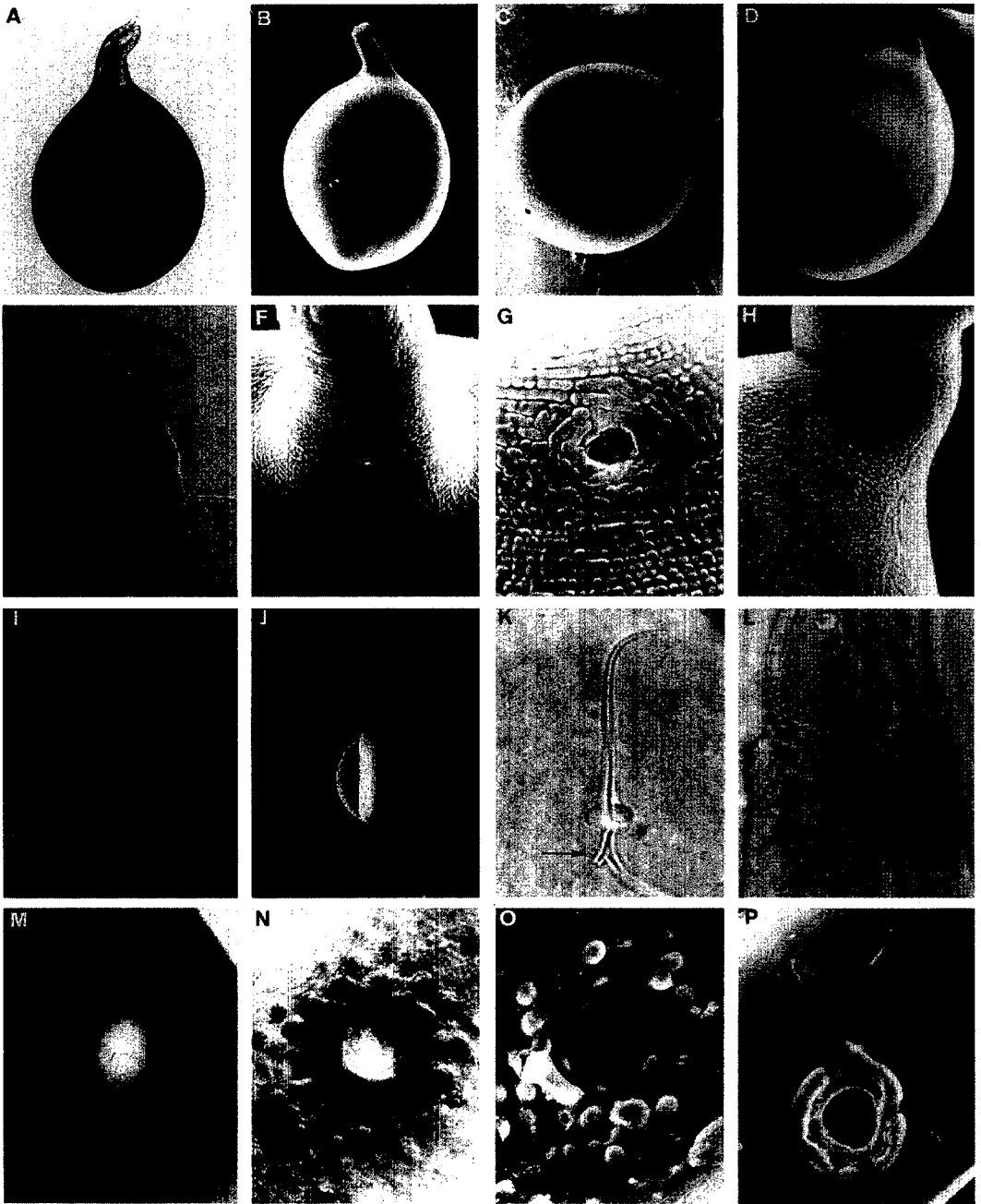


FIG. 1. General morphological characters of *Globodera tabacum* spp. females and cysts. GTT = *G. t. tabacum*; GTV = *G. t. virginiae*; GTS = *G. t. solanacearum*. LM = light microscopy; SEM = scanning electron microscopy. A) GTT-1 female, LM. B) GTV-6 cyst, SEM. C) GTT-1 cyst, SEM. D) GTS-1 cyst, SEM. E) GTT-1, female anterior region, LM. F) GTS-6, cyst neck and excretory pore, SEM. G) Excretory pore, detail magnified from F. H) GTS-9, cyst, SEM, detail of base of neck showing cuticular ridges and tubercles. I) GTS-1, stylet and median bulb lumen lining, SEM. J) Median bulb lumen lining, magnified from I. K) GTS-1 stylet showing DEGO (arrow), LM. L) GTT-2 vesicles attached to lumen lining, LM. M) GTS-15, finger-shaped bullae inside fenestra, LM. N) GTS-5, round bullae inside fenestra, SEM. O) Same as N, but LM. P) GTV-5, V-shape structure pointing to anus and thickening surrounding inside of fenestra, SEM.

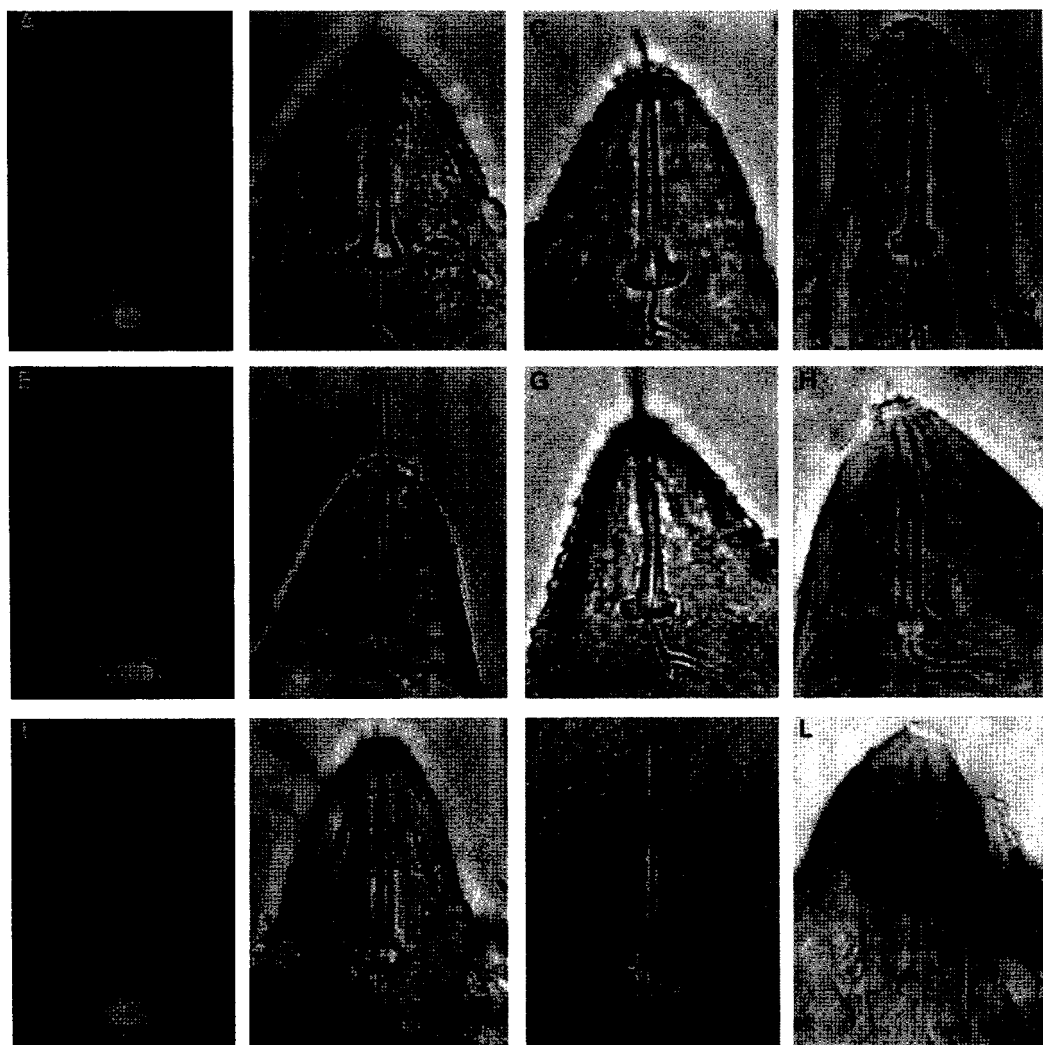


FIG. 2. Morphology of the anterior region of females of *Globodera tabacum* ssp. GTT = *G. t. tabacum*; GTV = *G. t. virginiae*; GTS = *G. t. solanacearum*. LM = light microscopy; SEM = scanning electron microscopy. B-D; F-H; and J-L, lateral view, LM. A) GTT-1 extracted stylet, SEM. B) GTT-2. C) GTT-1. D) GTT-2, E) GTV-1, extracted stylet, SEM. F) GTV-11. G) GTV-1. H) GTV-11. I) GTS-1, extracted stylet, SEM. J) GTS-10. K) GTS-1. L) GTS-1.

or valleys by LM. In LM, particularly with differential interference contrast microscopy, the image can appear inverted because of the optical illusion caused by directional lighting. The terminal area is circumfenestrated with a round to elliptical fenestra. Young cysts may have remnants of vulval tissue as well as perineal tubercles. Fully matured cysts show none of these structures; the fenestra is a hole from which the J2 emerge. Dark bodies

resembling the bullae found in some species of *Heterodera* were rarely present in the three subspecies (Fig. 1M-P). Both round (Fig. 1N,O) and finger-shaped bullae (Fig. 1M) were observed inside the terminal area, around the fenestra. A thick ring of cuticle, which may have been formed by the coalescence of the bullae, was present in some specimens (Fig. 1P). Inside the cyst wall, a conspicuous V-shaped groove marks the anus (Fig. 1P).

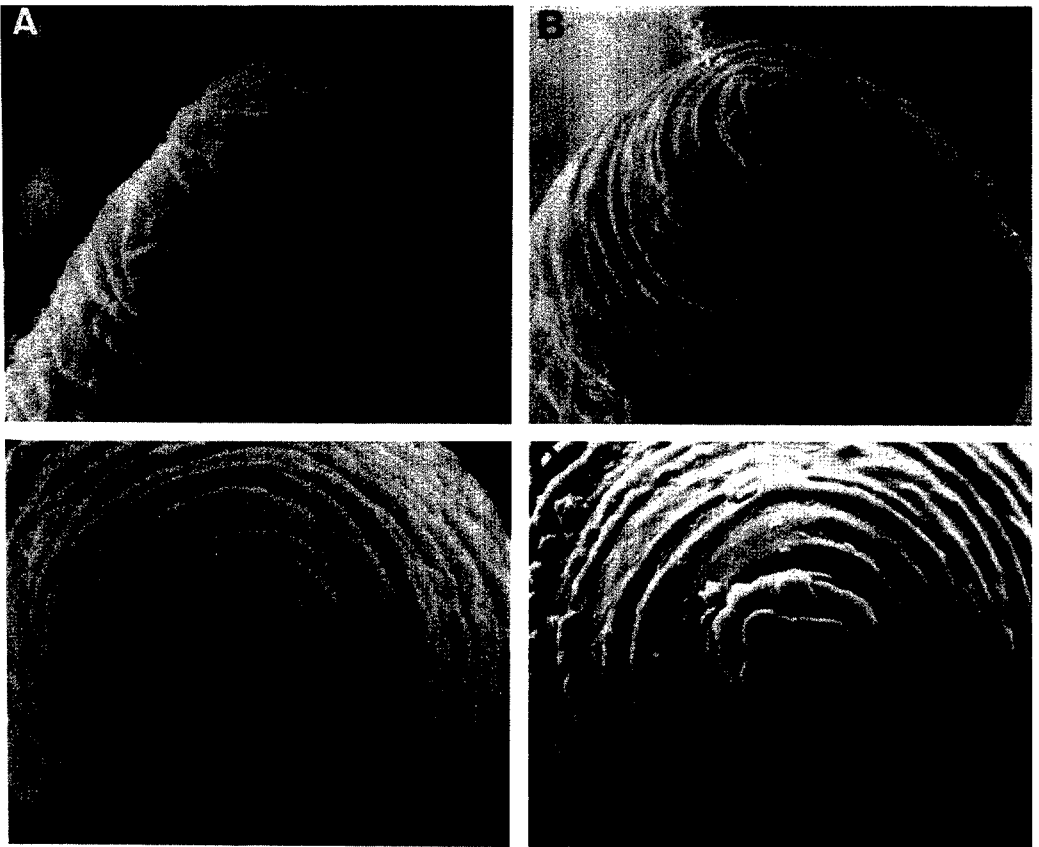


FIG. 3. Anterior region of females of *Globodera tabacum tabacum*, as seen in oblique (A, B) and face (C, D) SEM views. A, C) GTT-5. B, D) GTT-5.

The morphology of the ridges between the anus and the vulva is fairly characteristic for each subspecies. However, this character is variable among and within isolates of the same subspecies. In GTT the ridges are tightly packed and usually oriented in parallel rows perpendicular to the vulva–anus axis (Fig. 7A,D). In some specimens, however, the ridges are not parallel and are wider apart (Fig. 8A). Usually the anus is closer to the fenestra in GTT than in GTV and GTS, and it is also flanked by parallel ridges (Fig. 7A,D). In several specimens, the ridges around the anus are circular (Fig. 8A,G). Also, some specimens have a large crescent-shaped tail region just above the anus (Fig. 8D), although atypical for the subspecies.

In GTV the ridges between the fenestra

and anus usually are compacted and typically form a maze-like pattern (Fig. 7B,E), often extending to adjacent areas around the anus and the fenestra. In some variant specimens, the ridges may be wider apart, as in GTS (Fig. 8B). Some specimens of GTV also have parallel ridges in the anus–fenestra region similar to that of GTT (Fig. 8B). The anus of GTV is usually further from the fenestra than in GTT, but is difficult to locate because it is obscured by adjacent grooves and ridges. Usually the tail region is vague and not crescentic (Fig. 8E).

The typical cyst pattern for GTS has large, wide grooves between the ridges and no maze-like pattern of ridges between the anus and fenestra (Fig. 7C,F). Variants of this pattern, however, occur in all isolates. In some, parallel, tight ridges are present

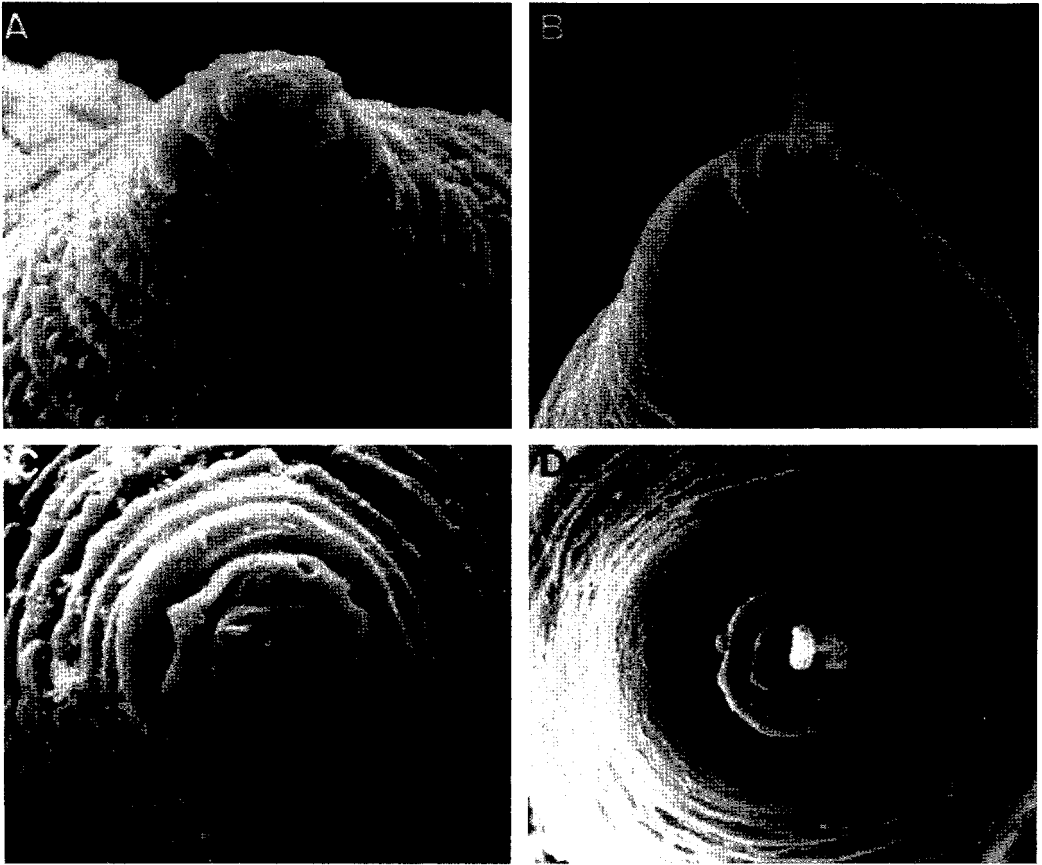


FIG. 4. Anterior region of females of *Globodera tabacum virginiae*, as seen in oblique (A, B) and face (C, D) SEM views. A, C) GTV-11. B, D) GTV-11.

in the anus-fenestra region similar to that of GTT, or the ridges run in the same direction of the anus-fenestra axis (Fig. 8I). The anus is usually separated from the ad-

jacent grooves in the center of a small anal basin (Figs. 7C,F;8F), and the tail region is usually crescent-shaped (Fig. 7C). None of the specimens of any of the isolates of GTS

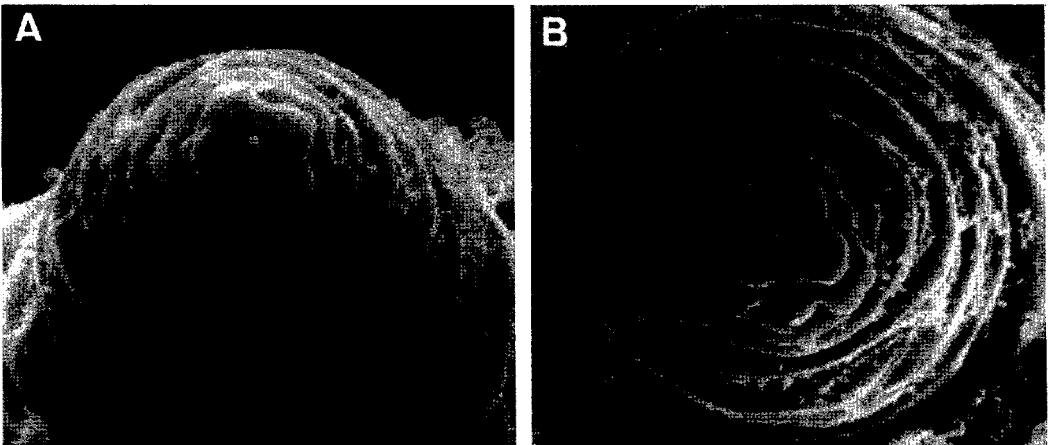


FIG. 5. Anterior region of females of *Globodera tabacum solanacearum*, as seen in oblique (A) and face (B) SEM views. A) GTS-10. B) GTS-10.

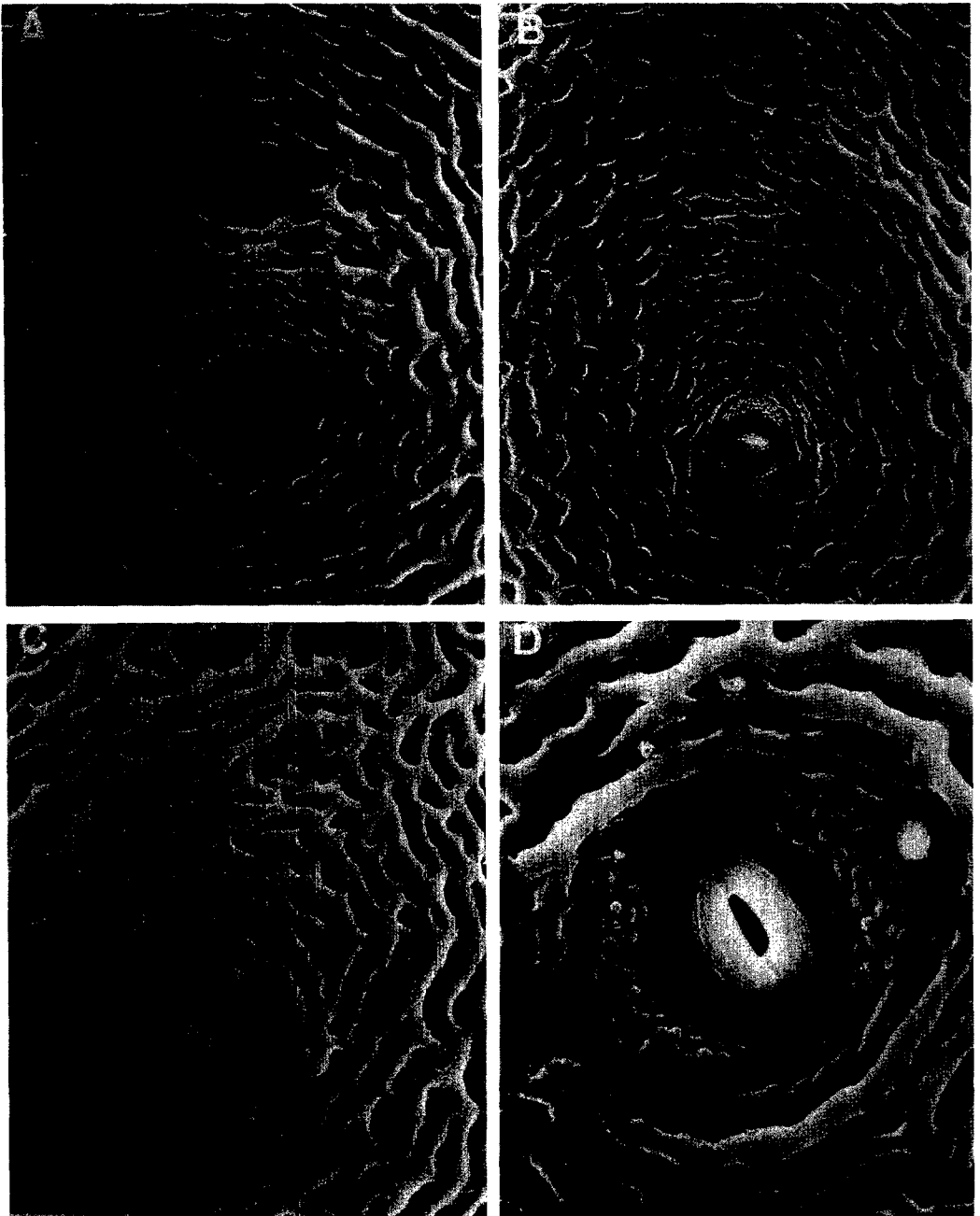


FIG. 6. SEM observations of the terminal area of females of *Globodera tabacum* ssp. GTT = *G. t. tabacum*; GTV = *G. t. virginiae*; GTS = *G. t. solanacearum*. A) GTT-1. B) GTV-1. C) GTS-1. D) Detail of the fenestra, magnified from C.

had parallel ridges running across the anal region.

DISCUSSION

Miller and Gray (9) used the area between the anus and fenestra to distinguish

Heterodera tabacum (=GTT), *H. virginiae* (=GTV), and *H. solanacearum* (=GTS). They distinguished GTS from the other two subspecies by the presence of widely spaced grooves in the terminal area. Although common in this subspecies, vari-

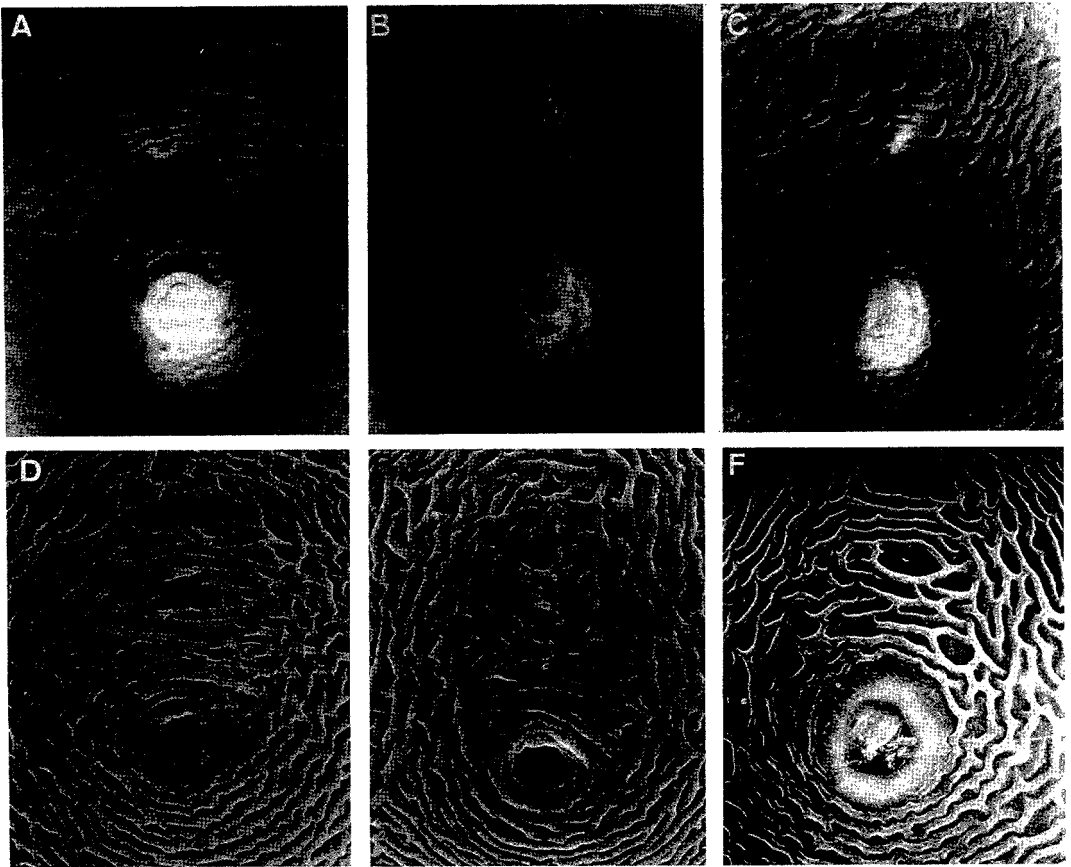


FIG. 7. LM (A–C) and SEM (D–F) observations of the terminal area of cysts of *Globodera tabacum* spp (typical cases). GTT = *G. t. tabacum*; GTV = *G. t. virginiae*; GTS = *G. t. solanacearum*. A) GTT-1. B) GTV-11. C) GTS-1. D) GTT-3. E) GTV-6. F) GTS-1.

ants with more closely spaced cuticular ridges are often found. This variability is implicitly accepted by the expression “presence of highly variable grooves in the terminal area” (9). The authors in a previous report distinguished GTV from GTT by maze-like grooves and ridges between the anus and fenestra in GTV compared to a non maze-like pattern in GTT (9). Our observations support the absence of maze-like ridges in GTT, but in some variants of GTV, parallel ridges occurred in the anus–vulva area (Fig. 8B,E). Miller and Gray (9) also distinguished the three subspecies by cyst and fenestral shape. Cysts are globose in GTT and elliptical in GTV and GTS. The fenestra is barrel-shaped with convex ends in GTS, circular to elliptical in GTV, and elliptical with obtuse ends in GTT.

Our observations indicate that the globose shape of the cyst is typical and consistent among all the GTT isolates by contrast to the more elongated (elliptical) shape in GTV and GTS. Shape of the cyst is the most reliable character for distinguishing GTT from GTV and GTS.

Green (3) observed several *Globodera* species with the SEM. Based on single isolates only, he found that the anus was near the middle of the terminal area in GTT, which resulted in relatively small anus to fenestra distance. Also, the ridges between anus and vulva were parallel and few ridges (6–7) were forked and rarely joined. The anal grooves were parallel. In GTS and GTV, the anal grooves formed a spiral, and the anus was closer to the tail tip. GTS had an anal area usually separated

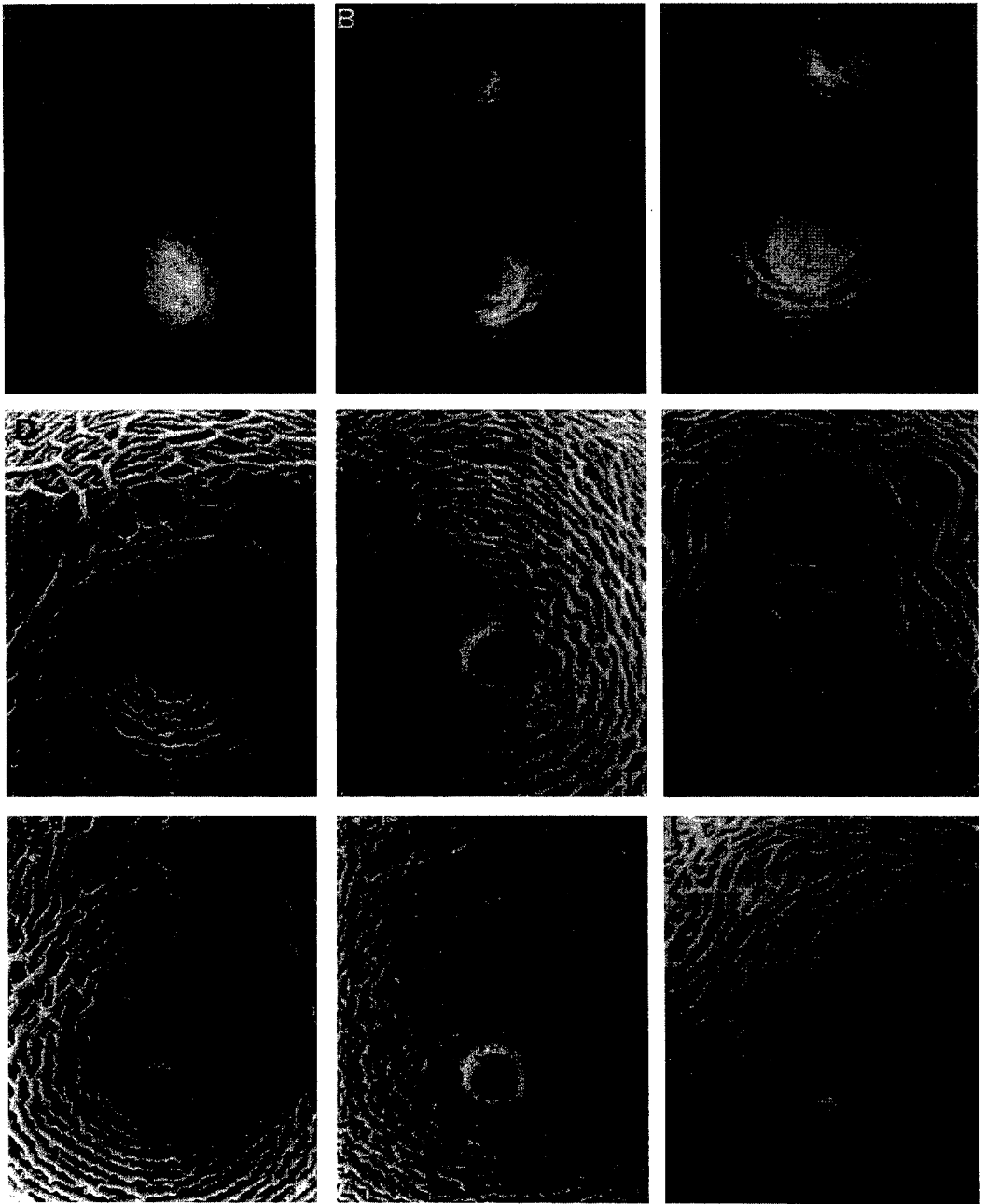


FIG. 8. LM observations of the terminal area of cysts of *Globodera tabacum* spp (variant cases). GTT = *G. t. tabacum*; GTV = *G. t. virginiae*; GTS = *G. t. solanacearum*. A) GTT-2. B) GTV-1-X. C) GTS-10. D) GTT-1. E) GTV-1-X. F) GTS-9. G) GTT-3. H) GTV-11. I) GTS-8.

from adjacent grooves, whereas in GTV it was continuous. The tail tip in GTS was large and crescentic, but in GTV it was indistinct or small and circular. Our observations of several isolates showed considerably more variability in the three subspe-

cies than reported by Green (3). The position of the anus relative to the fenestra was variable in comparisons of several isolates of the same subspecies. In GTT isolates, the anus was closer to the fenestra than some isolates of GTV and GTS, but

TABLE 2. Most important female and cyst characters for distinguishing *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS).

	Females		Cysts		
	Perineal tubercles	Stylet knobs	Shape	Anal-fenestra ridges	Anus
GTT	Clumped	Sloping backwards	Round	Parallel ridges	Distinct
GTV	Individual	Dorsal knob shaped like a "Dutch shoe"	Ovoid/ellipsoid	Maze-like pattern	Small indistinct
GTS	Individual	Moderately sloping backwards	Ovoid/ellipsoid	More widely separated	Distinct

not all. The ridges were typically parallel in the anus region of GTT, but were variable. In GTS, the anus was generally separated from the surrounding grooves, whereas in GTV the anus was more continuous with the grooves; in some cases it was difficult to locate. In GTS the tail region was often crescentic, but not in GTV and GTT.

Mulvey (14) distinguished GTS from GTV and GTT by a distinct circumfenestral area in most cysts; GTV has a maze-like pattern of lines between anus and vulva unlike GTT. In addition GTT has clumped tubercles, and in GTV and GTS they are more discrete. We found that the circumfenestral area is distinct in all isolates of all subspecies and does not seem to be characteristic of only GTS. We agree that the perineal tubercles are more clumped in GTT, particularly in white females, even though sometimes it is difficult to distinguish GTS and GTV from GTT by this character alone. The tubercles were distinct in many specimens of GTT. These tubercles may originate from transformation of the nearby ridges. Othman and Baldwin (15) reported the existence of tubercles on the neck region of females of all *Globodera* species. We have noticed that they occur frequently, but not always. The tubercles appear to form in the basal region of the neck from annules that fragment.

The most reliable characters for identification of these subspecies are the shape of the female stylet knobs, the maze-like pattern in the terminal area, and the small almost inconspicuous anus in GTV (Table

2). In GTT, the shape of the cyst is round, the ridges in the anal region are parallel, and the perineal tubercles are more clumped. In GTS, ridges in the terminal area are more widely separated, the anal region is distinct, and the tail region is crescent shaped. Future research on the development biology of these nematodes may clarify how the observed morphological characters change over time.

The taxonomic status of this complex has not been totally clarified. Even though Stone (16) proposed their status as subspecies, which was recently confirmed by Mugniery et al. (13) and Mota and Eisenback (12), not all authors agree. The three subspecies interbreed and produce viable hybrids (6). Recent developments on the morphology of some of the hybrids formed between GTV and GTS confirm their subspecific status (13). GTS has been suggested to be a junior synonym of GTV (13), but GTT was not included in the study. The morphology as well as the morphometrics of J2 and males failed to provide useful characters to distinguish these subspecies (12). The differences in the white females and cysts of the TCN complex observed in this study are quite subtle, except for the general shape, which also correlates with the morphometrics of the length/width ratio (12). Also, variants within the isolates of a particular subspecies have characters that are typical of other subspecies.

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